5-2019

Transcriptomic Analysis of Honey Bees Exposed to Organosilicone

Matthew Brent Thompson
Utah State University

TRANSCRIPTOMIC ANALYSIS OF HONEY BEES EXPOSED TO ORGANOSILICONE

by

Matthew Brent Thompson

Thesis submitted in partial fulfillment of the requirements for the degree

of

University Honors

in

Biology
in the Department of Biology

Approved:

Capstone Mentor Dr. Karen Kapheim	**Departmental Honors Advisor** Dr. Kimberly Sullivan

Director of University Honors Program
Dr. Kristine Miller

UTAH STATE UNIVERSITY
Logan, UT

May 2019

Abstract

Honey bees play an important role in agriculture and the decline of honey bee populations worldwide has generated concern. While the application of pesticides in agricultural settings is often implicated in the deterioration of honey bee population health, pesticide applications contain more than just pesticides; they also contain adjuvants that may have detrimental effects to bee health. One known effect of one type of adjuvant is the suppression of immunity-related genes and consequent increase of viral load in larvae. We investigate the effects of one class of adjuvant, organosilicone surfactants (OSS), on adult honey bee health. In a laboratory based bioassay, adult honey bees were fed various concentrations of an OSS (Xiameter® OFX-0309), alone and with a pesticide (Alticor®) and a fungicide (Tilt®). While survival of the bees was not affected by feeding regimes, bees ate significantly less diet on average if they were fed OSS at certain concentrations—indicating that bees that are exposed to these chemicals may suffer due to low food consumption. We then extracted and sequenced RNA from the bioassay bees to determine transcriptome profiles in bees from each feeding treatment. We found significant downregulation of some genes involved in metabolism in response to the pesticides and upregulation of a cytochrome P450 in response to the pesticides and OSS. In response to adjuvant exposure, serine protease snake was upregulated—a protein involved in innate immunity via activation of the Toll receptor and Rel pathway. OSS appears to interact synergistically with pesticides to exacerbate deleterious changes in gene expression.

Acknowledgements

First of all, I would like to thank Dr. Ellen Klinger for her tireless help and guidance in the lab. I also wish to thank Dr. Karen Kapheim for her invaluable instruction and advice with data analysis, and Dr. Diana Cox-Foster for guiding and motivating me through the entire project. I am very grateful to the USDA-ARS Pollinating Insect Research Unit for providing funding, a place to conduct this research, and great company. Finally, I would like to thank my friends and family for their continual support.

Contents

Figures and Tables

Introduction

Honey bees play a critical role in agriculture, contributing an estimated $15 billion to the U.S. economy alone (Calderone 2012). The decline in managed honey bee populations in recent years threatens crop production (Seitz et al. 2015). While no clear cause of this decline has been determined, several factors likely contribute to it including parasite infestation, pathogen infection, poor nutrition and pesticide exposure (Spivak et al. 2011).

Due to the social nature of honey bee colonies, pathogens and pesticides encountered in the environment by foragers are readily introduced into the colony via pollen and nectar. This facilitates systemic presence of pathogens and pesticides to all members of the colony, including nurse bees and brood (Rumkee et al. 2017, Calatayud-Vernich et al. 2018). There is evidence that pesticides and pathogens interact synergistically, furthering harm to honey bees. In particular, certain pesticides have been shown to impair honey bee immunity, consequently increasing viral load (DeGrandi-Hoffman et al. 2013, Di Prisco et al. 2013, Alburaki et al. 2015, Degrandi-Hoffman et al. 2015). Indeed, the effects of pesticides on non-target species have been heavily investigated, leading to their regulation and monitoring (Ware 1980). Much less research, however, has been conducted to investigate the effects of non-regulated, non-monitored chemicals used in pesticide applications such as adjuvants.

Adjuvants are chemicals that are mixed with pesticides to increase the efficacy of the active ingredient. One particular adjuvant, organosilicone (OSS), is commonly used in agricultural settings. OSS is a surfactant, added to pesticide batches to facilitate better spread of pesticides to crops (Ryckaert et al. 2008). The production of OSS has dramatically increased over the past several decades, with currently over a billion pounds of OSS produced per year for agricultural and other industrial purposes (Chen et al. 2012). OSS is widely used in annual

almond pollination in the Central Valley of California (Rücker and Kümmerer 2015, Chen et al. 2018). Approximately two-thirds of the managed honey bee colonies in the U.S. are involved in this pollination event every year, and consequently exposed to OSS and other agrochemicals (Calderone 2012). This wide-reaching exposure to OSS may help explain the decline in honey bee colonies across the nation.

While OSS is largely considered a biologically inert ingredient in pesticide applications, evidence of its deleterious effects on non-target species is accumulating. For example, OSS causes learning impairments in honey bees (Ciarlo et al. 2012). Fine et al. (2017) demonstrated that, in honey bee larvae, OSS increases the presence of Black Queen Cell Virus and decreases expression of certain Toll-7 receptor genes—which are involved in detoxification pathways. Other research suggests that the blend of adjuvants with pesticides increases toxicity to honey bees (Mullin et al. 2015). Previous research has demonstrated that OSS adjuvants facilitate higher viral loads in larval honey bees.

We further investigated the response to OSS exposure in adult honey bees by documenting how OSS interacts with pesticides to influence feeding behavior, mortality, and gene expression. We conducted a bioassay and subsequent transcriptomic analysis of adult honey bees exposed to OSS and asked the following questions:

1) Does exposure to the adjuvant OSS alone or with pesticides increase mortality or alter feeding behavior in adult honey bees?
2) Does exposure to OSS alone or with pesticides alter expression of genes involved in detoxification pathways?
3) How does gene expression in response to OSS differ from that in response to pesticides?

Methods

Bioassay. 36 cohorts of 10 adult honey bees (*Apis mellifera*) were collected from frames of a healthy, privately owned hive in Newton, Utah. Each cohort was placed in a plastic cup with filter paper covering the bottom and a modified syringe attached for delivering sugar water for bee consumption (Fig. 1). Treatments were prepared in 50% sugar water and included control, three concentrations of adjuvant (OSS: Xiameter® OFX-0309), pesticide (Alticor® and Tilt®), and combination (adjuvant medium and pesticides) (Table 1). Each cohort received 3 mL of one of the six treatments (or control) via feeder syringe. The feeder syringes were refilled with their respective treatment up to 3 mL daily. Consumption and mortality data were recorded. After 7 days of treatment, all bees were frozen with liquid nitrogen and stored at -80°C.

Table 1. Bees were fed one of six treatments of control, pesticide and/or adjuvant.

Treatment	Ingredients
Control	N/A
Adjuvant low	OSS (40 ppb)
Adjuvant medium	OSS (1 ppm)
Adjuvant high	OSS (10 ppm)
Pesticide	Alticor® (3 ppm) and Tilt® (150 ppb)
Combination	Alticor® (3 ppm), Tilt® (150 ppb), and OSS (1ppm)

Figure 1. Cohorts of 10 adult honey bees were kept in plastic cups for 7 days. Treatments were delivered via sugar water solution using a syringe. A piece of wax was placed in the container to give the bees a resting place.

from each replicate of the control, combination, pesticide, and medium adjuvant concentration (1 ppm) cohorts were randomly selected and decapitated on dry ice under sterile conditions. RNA was extracted from the combined abdomens and thoraces of the bees with the following protocol. The frozen samples were placed in a 2 mL tube with metal beads and homogenized for 1 minute at 1500 rpm. 1 mL of Trizol was immediately added to the tubes, then they were homogenized again for 30 seconds at 1500 rpm. After 5 minutes of incubation at room temperature, 200 µl of chloroform was added to the samples, followed by centrifuging for 15 minutes at 12,000 x g and 4 °C. From the aqueous phase of the sample, 450 µl was transferred to a new 1.5 mL tube. To isolate the RNA, 0.5 mL of 100% isopropanol was added, followed by incubation at -20 °C for >1 hour. The supernatant was then removed from the tube, leaving only the RNA pellet. The pellet was then washed with 75% etOH, allowed to dry, and resuspended in 50 µl RNase-free water. The RNA samples were stored at -80 °C.

Library Preparation & Sequencing. A library was prepared for each RNA sample (24 total) with Illumina's TruSeq stranded mRNA library preparation kit with the following modifications: in lieu of treating the samples with an oligoDT column, samples were treated with the RiboZero kit and DNase I, following their respective protocols. The RiboZero kit removes ribosomal RNA (rRNA) from the sample, and DNase digests DNA in the sample. Library quality and concentration were measured via tapestation at Utah State University's Center for Integrated Biosystems with 1 µl of each library (Supplemental Table 1). Library pooling and sequencing was performed at the Huntsman Cancer Institute High-Throughput Genomics and Bioinformatic Analysis Center. Treatments were labeled as follows: A: combination, B: pesticide, C: control, D: adjuvant. Due to high rRNA estimations, libraries produced from cohorts C4 and D6 were omitted from sequencing.

Differential Expression Analysis. Demultiplexing of sequencing output was performed at the Huntsman Cancer Institute High-Throughput Genomics and Bioinformatic Analysis Center. The quality of the data was assessed with FastQC. The resulting sequences were trimmed and low-quality sequences omitted with Trimmomatic using a MAXINFO operation with a target read length of 92 and strictness of 0.5. We also clipped the Illumina adapter sequences. We then aligned reads to the Amel_HAv3.1 honey bee genome (Wallberg et al. 2018) with HISAT2 (Kim et al. 2015) using paired and forward strandedness settings. All other settings were kept at defaults. Counting of the aligned reads was performed with HTseq count (union mode) (Anders et al. 2015). Differentially expressed genes were determined using the Bioconductor R package edgeR (Robinson et al. 2010) with the generalized linear model. Only genes with greater than 10 cpm in 2 or more samples were kept for the analysis. Sample A2 was excluded from the analysis due to high rRNA counts. Those genes with an FDR <0.05 were considered to be differentially expressed.

Results

Mortality and Consumption. Mortality during the bioassay among all cohorts of bees was negligible; only 2 bees died over the 7 days. Using a generalized linear model, we found a significant effect of treatment ($F=6.56$, $p<0.001$) and day ($F=8.04$, $p<0.001$) on sugar consumption per bee. Bees treated with adjuvant medium, adjuvant high, and pesticide treatments consumed significantly less food than control bees (Fig. 2 & Fig. 3).

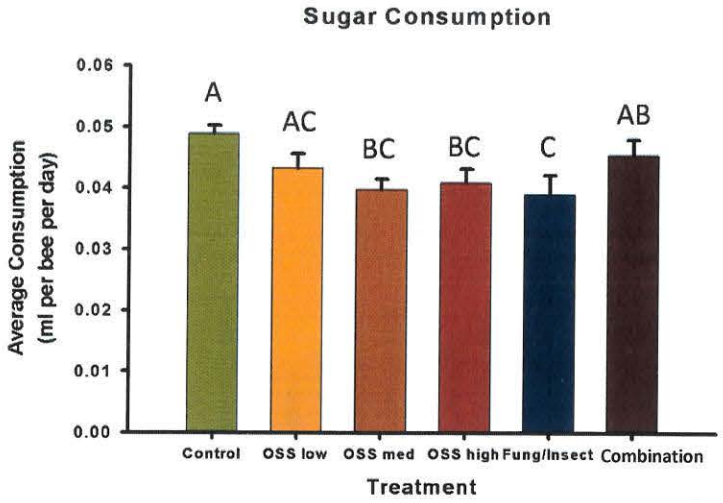

Figure 2. Bees exposed to moderate and high adjuvant concentrations showed significantly less feeding than control. Significant differences are indicated by lettering above the bars; treatments that do not share a letter are significantly different.

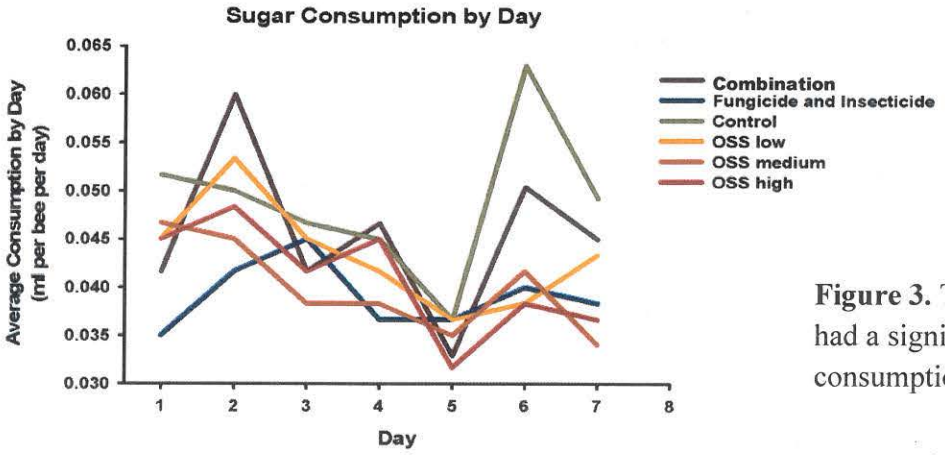

Figure 3. The day of treatment had a significant effect on sugar consumption.

Differential Expression. The total number of reads generated from sequencing was 430,398,414 with an average of 19,563,564.27 +/- 2570880.211 (s.d.) per sample (Fig. 4). After filtering and aligning to the Amel HAv3.1 honey bee genome, 207,254,923 unique exon hits were generated with an average of 9,420,678.318 +/- 1,9758,82.846 (s.d.) per sample. These were used in statistical analyses. Of the differentially expressed genes identified when including all replicates in the analysis, the majority coded for rRNA due to considerably higher rRNA counts in A2 (Fig. 6). To remedy this, the analysis was repeated without A2.

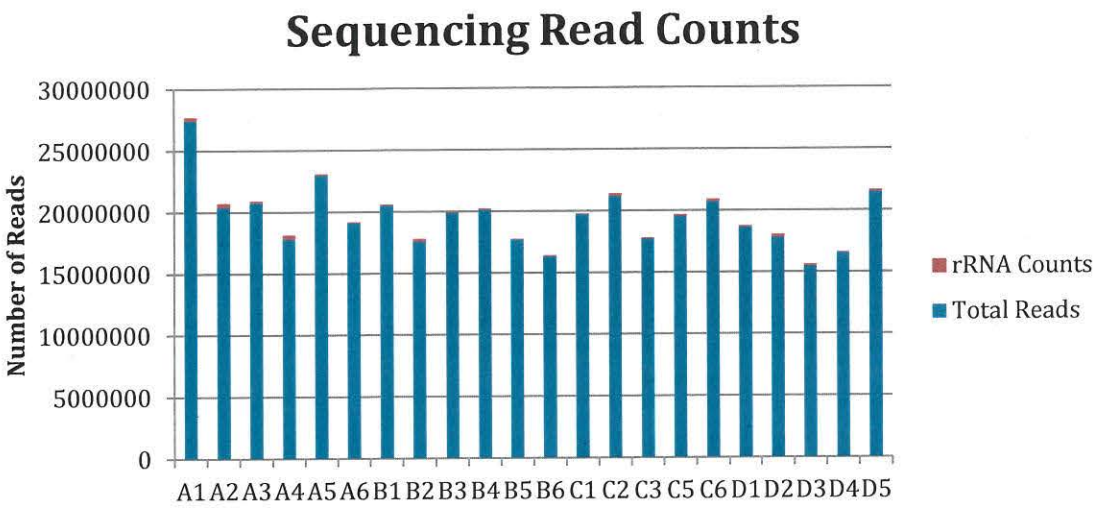

Figure 4. The number of reads produced from sequencing for each replicate. Red indicates the proportion of the total reads that aligned to a gene coding for rRNA.

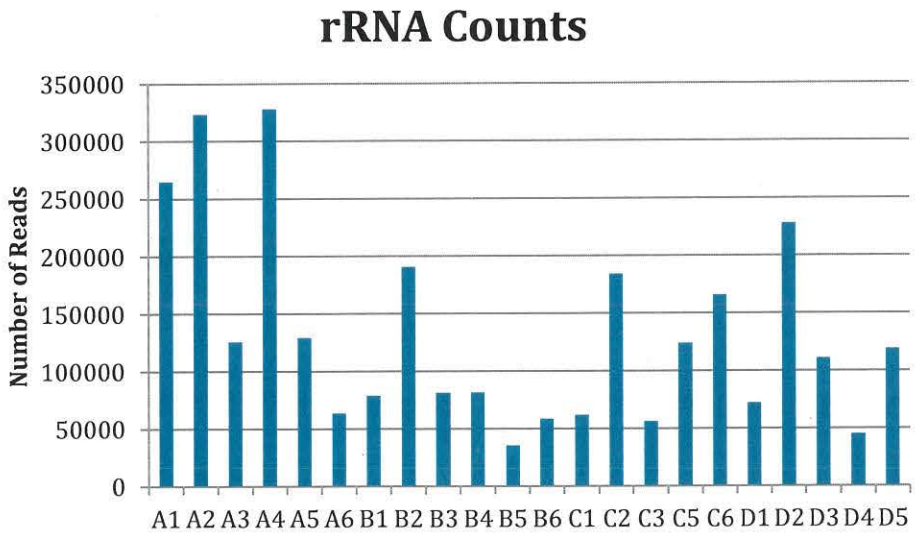

Figure 5. The number of reads that aligned to a gene coding for rRNA (red bars from fig. 4).

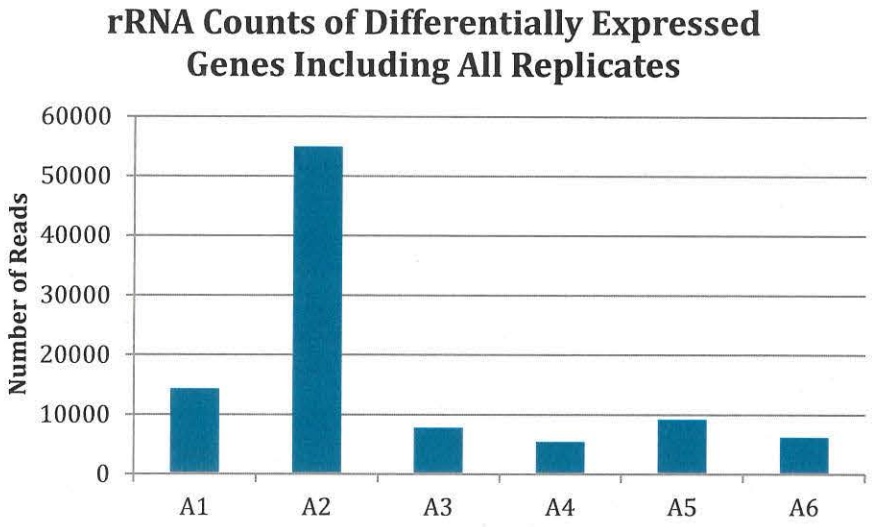

Figure 6. Replicate A2 had significantly higher counts of rRNA genes that were differentially expressed when all replicates were included in the analysis.

The number of differentially expressed genes was fairly consistent across the three treatments compared to control (Fig. 7, Supplemental Table 2). Bees exposed to the combination treatment (A) had 8 differentially expressed genes while those exposed to only pesticides (B) had 5 differentially expressed genes, and those exposed to adjuvant alone (D) had 5 differentially expressed genes. Three of the 8 genes differentially expressed in the combination treatment were also differentially expressed in the pesticide treatment. These three genes were all NADH dehydrogenase subunits (ND2, ND4, and ND6) and were all downregulated in both treatments. Additionally, a cytochrome P450 gene (LOC725087) was upregulated in bees exposed to the combination treatment. In bees exposed to adjuvant alone, a gene coding for serine protease snake (LOC724250) was upregulated, as well as a cytochrome c-type heme lyase gene (LOC408968).

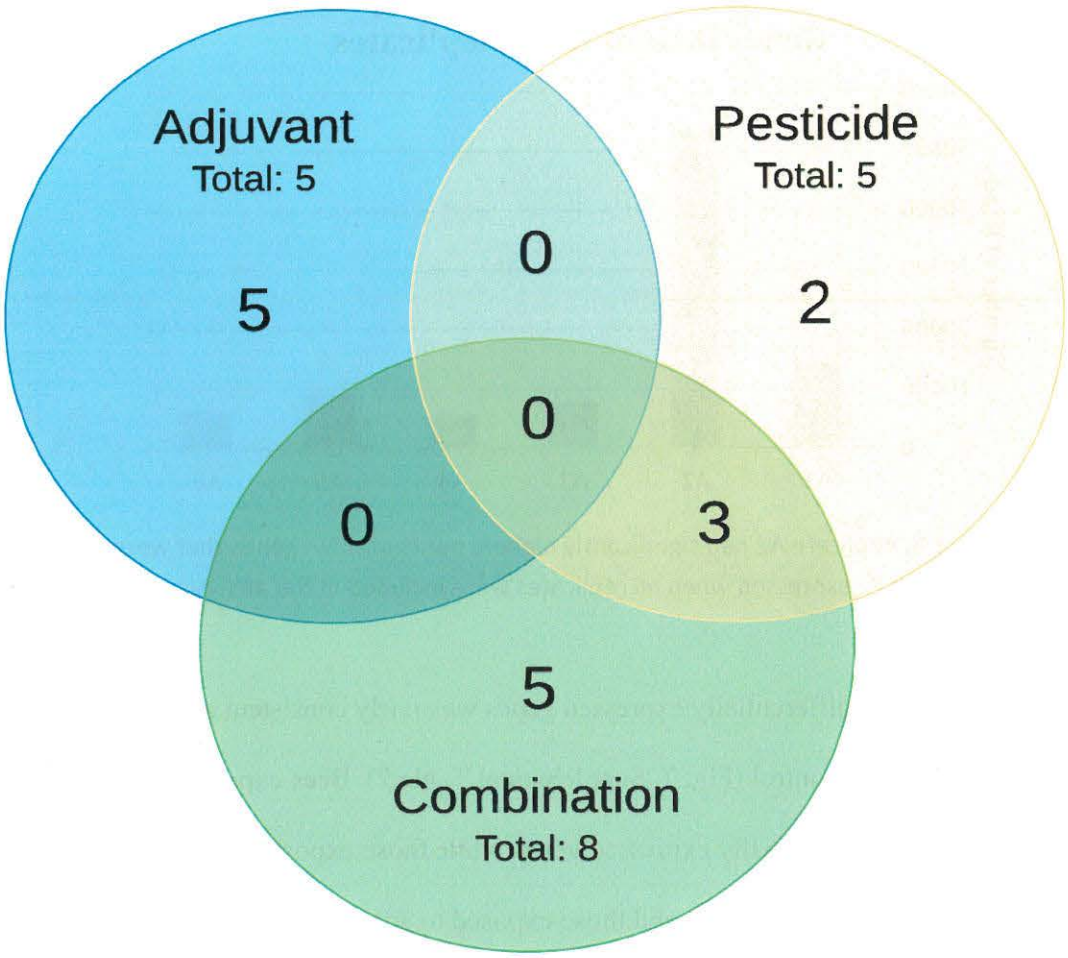

Figure 7. The number of differentially expressed genes compared was fairly consistent across treatments. Combination treatment and pesticide treatment shared 3 differentially expressed genes. Adjuvant treatment shared no differentially expressed genes.

Discussion

The objective of this study was to investigate the effects of OSS adjuvant exposure on adult honey bee gene expression, as well as feeding behavior and mortality. In previous research, honey bees have been shown to reduce their feeding behavior when their food is contaminated with agrochemicals (Degrandi-Hoffman et al. 2015). Here we show that OSS adjuvants have the same effect when present at concentrations comparable to or even more dilute than that which foragers may easily encounter in agricultural settings (1 ppm and 10 ppm). During the

application of pesticides, the concentration of OSS in the tank mix may range from 300 ppm to 5000 ppm (Mullin et al. 2015). Mortality during this 7-day bioassay was negligible. This may be because bees ate less of the treated sugar water and consequently received less toxicity. Additionally, it is interesting to note that consumption across all treatments declined until day 5, after which there was a sharp increase in consumption on days 6 and 7 for most treatments. It may be important to evaluate the method of delivery of OSS. In North American hives, considerably higher concentrations of OSS are present in pollen than honey (Chen and Mullin 2013). Since our sugar water solution simulated nectar, delivering the OSS via pollen would more accurately simulate natural conditions, and potentially produce different feeding and mortality results.

The putative functional classes of differentially expressed genes from our results were highly variable. The NADH dehydrogenase subunits are likely involved in metabolism. The fact that they were all downregulated in two of the treatments containing pesticides suggests that pesticides may affect metabolism, in addition to suppressing immunity as found in previous studies (Di Prisco et al. 2013). The upregulation of a cytochrome P450 gene in the combination treatment is expected since cytochrome P450s are well known to be involved in detoxification of xenobiotics (Xu et al. 2013, Berenbaum and Johnson 2015). A similar gene was upregulated in honey bee brains infected with BQCV (Doublet et al. 2016). Of the genes differentially expressed in the adjuvant treatment, only *serine protease snake* appears to be directly involved in detoxification pathways. In *Drosophila melanogaster* the serine protease snake is involved in embryo development (Steen et al. 2010) as well as activation of the cytokine Spätzle and in turn the activation of the Toll receptor and Rel pathway—both of which are components of the innate immune system of insects (Lemaitre and Hoffmann 2007, Kanost and Jiang 2015). The cytochrome c-type heme lyase is likely involved in cellular respiration (Babbitt et al. 2014).

Differentially expressed genes were determined with the most conservative estimate of significance (FDR) and under strict parameters. This explains the limited number of differentially expressed genes that we found. This is important to note when comparing differentially expressed genes across treatments and may account for the fact that no differentially expressed genes were shared between the adjuvant treatment and either of the other treatments, despite the fact that one other treatment included adjuvants. It is important to note that there were 5 differentially expressed genes in the combination treatment that were not differentially expressed the adjuvant or pesticide treatments. This suggests that OSS may interact synergistically with pesticides to have a greater effect on gene expression.

To obtain a more comprehensive list of potentially differentially expressed genes, further statistical analyses will be conducted with modified significance parameters. Once we identify differentially expressed genes with the most functional relevance to detoxification, we plan to verify the changes in gene expression of these genes via quantitative polymerase chain reaction with RNA from the original bioassay bees. Additionally, we will conduct further examination of the sequence data to identify changes in positive-sense single-stranded RNA viruses across treatments.

Finally, the results of this experiment are constrained by the limited sample of bees used. Since the bees used for this study were collected from a frame inside the hive, they are likely almost all nurse bees. The transition from nurse bee to forager involves changes in expression of thousands of genes (Whitfield et al. 2006). Consequently, genetic expression in response to OSS adjuvants of honey bees of different social roles may vary dramatically. Thus, further research investigating these genetic consequences in honey bees of different social roles and different hives would be informative.

Though our results are limited and further research is needed, we support the existing literature that exposure to OSS appears to have deleterious effects on honey bees. This is evidentfrom the reduction in feeding behavior and altered gene expression in response to OSS. While no significant downregulation of detoxification genes was found, there was a significant downregulation of important metabolic genes. Importantly, many of the changes in gene expression appear to be a result of synergistic interactions between OSS and commonly used pesticides.

Reflective Writing

I had no idea what I was getting myself into when I started this project. I had been working at the USDA-ARS "Bee Lab" for several months when I decided I wanted to work on an independent project there. I worked (and still work) in the pathology lab helping with field experiments and doing molecular work to screen for viruses in honey bees. This piqued my interest in working with genetic material. I liked the idea of being able to find out what's going on at the micro world and how that affects the macro world. I also liked how universal genetics is (literally) and how easily skills with genetics transfer to different fields. Admittedly, my enthusiasm for honey bees was not profound in my past state of ignorance, but as I've become intimately familiar with their plight I've gained a significant appreciation for and genuine interest in the biology of honey bees and their general well-being.

Initially, I had no intention that this project I had in mind would turn into my capstone project. I just wanted a research project that I could have some ownership over. I knew I wanted to do something with genetics, but at that point I only had a rudimentary knowledge of conceptual genetics from a class I took. The nitty gritty molecular procedures and bioinformatic strategies that I now have at least a modest grasp on were then entirely foreign to me. I called Dr. Cox-Foster, my supervisor, to talk about starting an independent project, to which she responded enthusiastically.

Over the next few months, Dr. Cox-Foster, Dr. Klinger (another scientist at the Bee Lab), Dr. Kapheim (a USU faculty member), and I devised and organized a suitable project for me to work on. It took a while to nail down exactly what this was going to be. We talked about doing a metagenomic analysis of a bunch of dead bees from an unrelated experiment that took a turn for the worst to find out what killed them, but thankfully we realized that this would be very messy and decided against it. Coincidentally, around this time we were conducting a bioassay in the lab

looking at how organosilicone and pesticides interacted to affect honey bee health. We decided that I would take over this bioassay and take it a step further than was originally intended. From the bees that didn't die during the bioassay (which was most of them) I would sequence messenger RNA to find out how the chemicals they were exposed to were affecting their gene expression. This idea was exciting to me because, frankly, I had no idea that it was possible.

The learning curve for this project was immense. Starting out, I was at virtual ground zero. As I mentioned before, the only background knowledge I had with this sort of genetic analysis was a few introductory classes and limited lab work experience at the Bee Lab. I started reading piles of literature that Dr. Cox-Foster supplied me with in order to grasp the overall process of the project, but I essentially learned as I went. Thankfully, I was familiar with the first step in the process, RNA extraction, since I had extracted RNA from probably hundreds of samples by that point. The largest hurdles were turning the RNA into libraries to be sequenced and the analysis of the sequence data that followed.

By the end of the fall semester of my junior year, we had a collection of RNA samples and a tentative plan to sequence them. For the process of library preparation I am forever grateful for Dr. Klinger. She worked devotedly to help me through every step of the process. Much sweat and blood were put into those libraries (though not literally—that would make for poor sequencing). I even ended up sacrificing a spring break to finish them. But ultimately it was worth it.

Once we got the sequencing data back from the University of Utah (they had fancier equipment) and after I returned from an internship in North Carolina, I began the bioinformatics portion of the project. I thought the rest of the project would be smooth sailing after the sequencing was completed, but little did I know that bioinformatics would prove to be an equally

formidable foe. Thankfully, Dr. Kapheim and Dr. Cox-Foster provided instruction, direction, and much needed correction throughout the process and over numerous meetings. Early on in this process I focused primarily on using a software package pipeline which was new to all of us (Kallisto-Sleuth). Though we did figure out how to use it and got some (questionable) results from it, we decided to set it aside (though not entirely abandoning it) in favor of a more traditional pipeline, (HISAT2-HTseq-edgeR). This pipeline proved to be much more straightforward and more readily produced comprehendible results (likely just because of Dr. Kapheim's proficiency with it—this isn't intended to be a condemning review of Kallisto-Sleuth). While we've made significant progress with the sequence analysis (at least enough to write a capstone) there's still a considerable amount left to do.

A few months ago, I was struggling through the various software programs to analyze the sequence data and had not even begun writing this capstone. I was feeling particularly overwhelmed with it and I didn't think I was going to be able to finish my capstone. I even told Dr. Cox-Foster that I wasn't going to be able to complete it in time. Dr. Cox-Foster invited me to her office and helped me mentally work through the difficulties and encouraged me to at least try finishing. Thanks to her, I'm now completing this last part of my capstone (albeit very close to the deadline).

I remember on some honors contract somewhere I predicted that I would be completely finished with this project—analysis and all—within two semesters. It has now been four semesters and there's still a lot of work to do. Thankfully, there's plenty of time to do it—after I graduate. I plan to continue to work at the Bee Lab, helping to write up a publishable manuscript of this project.

References

Alburaki, M., S. Boutin, P.-L. Mercier, Y. Loublier, M. Chagnon, and N. Derome. 2015. Neonicotinoid-Coated Zea mays Seeds Indirectly Affect Honeybee Performance and Pathogen Susceptibility in Field Trials. PLoS One. 10: e0125790.

Anders, S., P. T. Pyl, and W. Huber. 2015. HTSeq--a Python framework to work with high-throughput sequencing data. Bioinformatics. 31: 166–9.

Babbitt, S. E., B. San Francisco, D. L. Mendez, G. S. Lukat-Rodgers, K. R. Rodgers, E. C. Bretsnyder, and R. G. Kranz. 2014. Mechanisms of mitochondrial holocytochrome c synthase and the key roles played by cysteines and histidine of the heme attachment site, Cys-XX-Cys-His. J. Biol. Chem. 289: 28795–807.

Berenbaum, M. R., and R. M. Johnson. 2015. Xenobiotic detoxification pathways in honey bees. Curr. Opin. Insect Sci. 10: 51–58.

Calatayud-Vernich, P., F. Calatayud, E. Simó, and Y. Picó. 2018. Pesticide residues in honey bees, pollen and beeswax: Assessing beehive exposure. Environ. Pollut. 241: 106–114.

Calderone, N. W. 2012. Insect Pollinated Crops, Insect Pollinators and US Agriculture: Trend Analysis of Aggregate Data for the Period 1992–2009. PLoS One. 7: e37235.

Chen, J., J. D. Fine, and C. A. Mullin. 2018. Are organosilicon surfactants safe for bees or humans? Sci. Total Environ. 612: 415–421.

Chen, J., and C. A. Mullin. 2013. Quantitative Determination of Trisiloxane Surfactants in Beehive Environments Based on Liquid Chromatography Coupled to Mass Spectrometry. Environ. Sci. Technol. 47: 9317–9323.

Chen, Y. P., J. J. Becnel, and S. M. Valles. 2012. RNA Viruses Infecting Pest Insects, pp. 133–170. *In* Insect Pathol. Elsevier.

Ciarlo, T. J., C. A. Mullin, J. L. Frazier, and D. R. Schmehl. 2012. Learning Impairment in Honey Bees Caused by Agricultural Spray Adjuvants. PLoS One. 7: e40848.

DeGrandi-Hoffman, G., Y. Chen, and R. Simonds. 2013. The Effects of Pesticides on Queen Rearing and Virus Titers in Honey Bees (Apis mellifera L.). Insects. 4: 71–89.

Degrandi-Hoffman, G., Y. Chen, E. Watkins Dejong, M. L. Chambers, and G. Hidalgo. 2015. Effects of Oral Exposure to Fungicides on Honey Bee Nutrition and Virus Levels. J. Econ. Entomol. 108: 2518–2528.

Doublet, V., R. J. Paxton, C. M. McDonnell, E. Dubois, S. Nidelet, R. F. A. Moritz, C. Alaux, and Y. Le Conte. 2016. Brain transcriptomes of honey bees (Apis mellifera) experimentally infected by two pathogens: Black queen cell virus and Nosema ceranae. Genomics data. 10: 79–82.

Kanost, M. R., and H. Jiang. 2015. Clip-domain serine proteases as immune factors in insect hemolymph. Curr. Opin. Insect Sci. 11: 47–55.

Kim, D., B. Langmead, and S. L. Salzberg. 2015. HISAT: a fast spliced aligner with low memory requirements. Nat. Methods. 12: 357–360.

Lemaitre, B., and J. Hoffmann. 2007. The Host Defense of *Drosophila melanogaster*. Annu. Rev. Immunol. 25: 697–743.

Mullin, C. A., J. Chen, J. D. Fine, M. T. Frazier, and J. L. Frazier. 2015. The formulation makes the honey bee poison. Pestic. Biochem. Physiol. 120: 27–35.

Di Prisco, G., V. Cavaliere, D. Annoscia, P. Varricchio, E. Caprio, F. Nazzi, G. Gargiulo, and F. Pennacchio. 2013. Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. Proc. Natl. Acad. Sci. 110: 18466–18471.

Robinson, M. D., D. J. McCarthy, and G. K. Smyth. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 26: 139–40.

Rücker, C., and K. Kümmerer. 2015. Environmental Chemistry of Organosiloxanes. Chem. Rev. 115: 466–524.

Rumkee, J. C. O., M. A. Becher, P. Thorbek, and J. L. Osborne. 2017. Modeling Effects of Honeybee Behaviors on the Distribution of Pesticide in Nectar within a Hive and Resultant in-Hive Exposure.

Ryckaert, B., P. Spanoghe, B. Heremans, G. Haesaert, and W. Steurbaut. 2008. Possibilities To Use Tank-Mix Adjuvants for Better Fungicide Spreading on Triticale Ears. J. Agric. Food Chem. 56: 8041–8044.

Seitz, N., K. S. Traynor, N. Steinhauer, K. Rennich, M. E. Wilson, J. D. Ellis, R. Rose, D. R. Tarpy, R. R. Sagili, D. M. Caron, K. S. Delaplane, J. Rangel, K. Lee, K. Baylis, J. T. Wilkes, J. A. Skinner, J. S. Pettis, and D. vanEngelsdorp. 2015. A national survey of managed honey bee 2014–2015 annual colony losses in the USA. J. Apic. Res. 54: 292–304.

Spivak, M., E. Mader, M. Vaughan, and N. H. Euliss. 2011. The Plight of the Bees †. Environ. Sci. Technol. 45: 34–38.

Steen, P. W., S. Tian, S. E. Tully, B. F. Cravatt, and E. K. LeMosy. 2010. Activation of Snake in a serine protease cascade that defines the dorsoventral axis is atypical and pipe-independent in Drosophila embryos. FEBS Lett. 584: 3557–60.

Wallberg, A., I. Bunikis, O. V. Pettersson, M.-B. Mosbech, A. K. Childers, J. D. Evans, A. S. Mikheyev, G. E. Robinson, H. M. Robertson, and M. T. Webster. 2018. A hybrid de novo genome assembly of the honeybee, Apis mellifera, with chromosome-length scaffolds.

bioRxiv. 361469.

Ware, G. W. 1980. Effects of pesticides on nontarget organisms, pp. 173–201. *In* Residue Rev. Springer New York, New York, NY.

Whitfield, C. W., Y. Ben-Shahar, C. Brillet, I. Leoncini, D. Crauser, Y. Leconte, S. Rodriguez-Zas, and G. E. Robinson. 2006. Genomic dissection of behavioral maturation in the honey bee. Proc. Natl. Acad. Sci. U. S. A. 103: 16068–75.

Xu, J., J. P. Strange, D. L. Welker, and R. R. James. 2013. Detoxification and stress response genes expressed in a western North American bumble bee, Bombus huntii (Hymenoptera: Apidae). BMC Genomics. 14: 874.

Supplemental Data

Supplemental Table 1. Library quality and concentration were determined via tapestation.

Sample Description	Size [bp]	Calibrated Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area
A1	299	6250	32200	100
A2	154	188	1880	3.03
A2	285	6020	32400	96.97
A3	294	11600	60600	100
A4	157	126	1230	0.81
A4	296	15400	80300	99.19
A5	286	11700	62800	100
A6	300	17800	91200	100
B1	282	4060	22200	100
B2 (1)	161	1180	11300	15
B2 (1)	294	6480	33900	82.2
B2 (2)	286	30700	165000	100
B3	163	295	2790	3.02
B3	333	9480	43800	96.98
B4	297	18600	96400	100
B5	313	15800	77700	100
B6	312	12200	60100	100
C1	156	140	1380	0.98
C1	300	14100	72500	99.02
C2	153	578	5820	4.5
C2	304	12300	62000	95.5
C3	149	947	9750	9.48
C3	275	8410	47100	84.21
C3	2918	631	333	6.32
C4	159	420	4070	3.33
C4	307	12200	60900	96.67
C5		6990		100
C6	162	374	3560	3.23
C6	320	11200	53900	96.77
D1	151	302	3080	3.45
D1	289	8290	44100	94.82
D2	160	521	5000	3.8
D2	278	13200	73200	96.2
D3	151	719	7330	5.8
D3	280	11700	64200	94.2
D4	156	523	5150	2.39
D4	284	21300	115000	97.61

D5	156	263	2600	1.53
D5	296	16200	84100	94.06
D6	50	15.5	472	0.07
D6	117	277	3650	1.17
D6	252	23400	143000	98.77

edgeR code

```
CountTable <- read.table("countData_sansA2.txt", header=TRUE)
annotation = read.table("edger_annotation.txt", header = TRUE)
groups = c("A", "A", "A", "A", "A", "B", "B", "B", "B", "B", "B", "C", "C", "C", "C", "C", "D", "D", "D",
"D", "D")
f <- DGEList(counts=CountTable,group=factor(groups), genes =annotation )
apply(f$counts, 2, sum)
keep <- rowSums(cpm(f)>10) >= 2
f <- f[keep,]
f$samples$lib.size <- colSums(f$counts)
f <- calcNormFactors(f)
design.mat <- model.matrix(~ 0 + f$samples$group)
colnames(design.mat) <- levels(f$samples$group)
f2 <- estimateGLMCommonDisp(f,design.mat)
f2 <- estimateGLMTrendedDisp(f2,design.mat, method="auto")
f2 <- estimateGLMTagwiseDisp(f2,design.mat)
fit <- glmFit(f2, design.mat)
lrt13 <- glmLRT(fit, contrast=c(1,0,-1,0))
lrt23 <- glmLRT(fit, contrast=c(0,1,-1,0))
lrt43 <- glmLRT(fit, contrast=c(0,0,-1,1))
topTags(lrt13, n=20)
topTags(lrt23, n=40)
topTags(lrt43, n=40)
```

Supplemental Table 2. Differentially expressed genes identified with edgeR. Treatments were compared to control condition. A = combination, B = pesticide, D = adjuvant, C = control. Genes with an FDR <0.05 were considered differentially expressed. logFC >0 indicates upregulation, logFC <0 indicates downregulation.

Treatment	geneID	logFC	logCPM	LR	PValue	FDR	description
A-C	gene12361	-2.4786436	5.726573	62.8305	2.25E-15	1.61E-11	NADH dehydrogenase subunit 2
A-C	gene12371	-2.4051365	3.54976	28.61199	8.84E-08	3.14E-04	NADH dehydrogenase subunit 6
A-C	gene12369	-1.5859067	4.90858	27.06933	1.96E-07	3.14E-04	NADH dehydrogenase subunit 4
A-C	gene12364	-3.6193069	2.229185	26.91451	2.13E-07	3.14E-04	ATP synthase F0 subunit 8
A-C	gene6738	-2.2318023	3.407872	26.85566	2.19E-07	3.14E-04	U2 spliceosomal RNA
A-C	gene5371	-10.494894	4.035586	22.35037	2.27E-06	2.71E-03	uncharacterized
A-C	gene12004	1.7610119	7.696782	17.69113	2.60E-05	2.66E-02	elongation of very long chain fatty acids protein AAEL008004
A-C	gene10507	4.9715996	6.212122	16.34834	5.27E-05	4.71E-02	probable cytochrome P450 6a14
A-C	gene6739	-1.2367502	7.491526	15.85113	6.85E-05	5.45E-02	U2 spliceosomal RNA
A-C	gene12352	2.9494464	9.138815	15.29815	9.18E-05	6.57E-02	small subunit ribosomal RNA
B-C	gene12361	-2.0322958	5.7265733	49.484038	2.00E-12	1.43E-08	NADH dehydrogenase subunit 2
B-C	gene12369	-1.5072483	4.9085801	27.490679	1.58E-07	5.65E-04	NADH dehydrogenase subunit 4
B-C	gene11416	-0.9810588	4.6244102	22.915465	1.69E-06	4.04E-03	uncharacterized
B-C	gene12371	-1.9352144	3.54976	21.641469	3.29E-06	5.88E-03	NADH dehydrogenase subunit 6
B-C	gene876	-2.263512	3.6740291	17.725906	2.55E-05	3.65E-02	uncharacterized
D-C	gene11388	1.3316002	3.845555	33.77353	6.19E-09	4.429E-05	serine protease snake
D-C	gene3059	1.1751236	4.280173	29.94316	4.45E-08	0.0001591	uncharacterized
D-C	gene10001	0.7086648	7.580166	21.81315	3.01E-06	0.0071657	protein unc-45 homolog B
D-C	gene8693	1.2136861	4.666937	18.06727	2.13E-05	0.0370946	SET and MYND domain-containing protein 4
D-C	gene6818	0.5226514	7.078169	17.6951	2.59E-05	0.0370946	cytochrome c-type heme lyase

Author's Biography

Matthew Thompson is graduating with a Bachelors degree in biology with minors in psychology and chemistry. His accomplishments as an undergraduate include being a part of the Honors program, an Undergraduate Research Fellow, and jumping into first dam in the middle of winter. After graduation, Matthew will continue working on this project for publication followed by graduate school at an unknown location in an unknown discipline.